THE LANCET Infectious Diseases

Supplementary webappendix

This webappendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Pritt BS, Mead PS, Johnson DKH, et al. Identification of a novel pathogenic Borrelia species causing Lyme borreliosis with unusually high spirochaetaemia: a descriptive study. *Lancet Infect Dis* 2016; published online Feb 5. http://dx.doi.org/10.1016/S1473-3099(15)00464-8.

Appendix (Web Extra Material)

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Supplemental Table 1. Novel Bbsl genospecies gene sequences deposited in GenBank*

Designation	Accession Number
EC10N1 *uvrA*	KM289011
EC10N1 *rplB*	KM289012
EC10N1 *recG*	KM289013
EC10N1 *pyrG*	KM289014
EC10N1 *pepX*	KM289015
EC10N1 *clpX*	KM289016
EC10N1 *clpA*	KM289017
ND132 *uvrA*	KM289018
ND132 *rplB*	KM289019
ND132 *recG*	KM289020
ND132 *pyrG*	KM289021
ND132 *pepX*	KM289022
ND132 *clpX*	KM289023
ND132 *clpA*	KM289024
WI133 *uvrA*	KM289025
WI133 *rplB*	KM289026
WI133 *recG*	KM289027
WI133 *pyrG*	KM289028
WI133 *pepX*	KM289029
WI133 *clpX*	KM289030
WI133 *clpA*	KM289031
MN14-1420 *uvrA*	KM877342
MN14-1420 *rplB*	KM877343
MN14-1420 *recG*	KM877344
MN14-1420 *pyrG*	KM877345
MN14-1420 *pepX*	KM877346
MN14-1420 *clpX*	KM877347
MN14-1420 *clpA*	KM877348
MN14-1420 *nifS*	KP972469
MN14-1420 16S rDNA	KP972468
MN14-1420 *flaB*	KR154295
MN14-1420 *ospC*	KR154297
MN14-1420 *rrf-rrl*	KR154296
MN14-1539 *uvrA*	KM877335
MN14-1539 *rplB*	KM877336
MN14-1539 *recG*	KM877337
MN14-1539 *pyrG*	KM877338
MN14-1539 *pepX*	KM877339
MN14-1539 *clpX*	KM877340
MN14-1539 *clpA*	KM877341
MN14-1539 16S rDNA	KP972466
MN14-1539 *nifS*	KP972467
MN14-1539 *flaB*	KR154292
MN14-1539 *ospC*	KR154294
MN14-1539 *rrf-rrl*	KR154293

* Sequences will be released by GenBank at time of manuscript publication. Sequence nomenclature (e.g. MN14-1538, WI133) represents the state from which the specimens were submitted and does not necessarily reflect the patient's state of residence.

Supplemental Table 2. Pairwise genetic distances of 7 concatenated housekeeping genes from tick (EC10N1) and human (ND132, W1133, MN14-1420, MN14-1539) (highlighted in blue) as compared to 18 Bbsl genospecies and 3 RF species. Pairwise genetic distances were calculated in MEGA 5.0 using the Kimura-2 parameter model. Percent genetic similarity is calculated using the formula: 100 − (genetic distance × 100).

Strains	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1. *B. afzelii* PKo																													
2. *B. americana* SCW-30h	0.085																												
3. *B. andersonii* 21123	0.082	0.056																											
4. *B. bavariensis* PZwi	0.064	0.080	0.081																										
5. *B. bissetti* DN127	0.080	0.060	0.063	0.077																									
6. *B. burgdorferi* B31	0.077	0.052	0.057	0.075	0.057																								
7. *B. burgdorferi* Z41293	0.077	0.051	0.054	0.076	0.059	0.017																							
8. *B. garinii* 20047	0.065	0.080	0.079	0.020	0.076	0.073	0.074																						
9. *B. kurtenbachii* IL96-255	0.083	0.064	0.066	0.079	0.038	0.060	0.062	0.079																					
10. *B. spielmanii* PsigII 27/6	0.066	0.088	0.088	0.076	0.087	0.082	0.082	0.077	0.089																				
11. *Borrelia* sp. SCW-30a	0.083	0.018	0.054	0.081	0.060	0.053	0.050	0.081	0.064	0.089																			
12. EC10N1 (tick, WI)	0.073	0.060	0.067	0.069	0.058	0.043	0.044	0.066	0.061	0.080	0.062																		
13. ND132 (Patient 2)	0.073	0.060	0.067	0.069	0.058	0.043	0.044	0.066	0.061	0.080	0.062	0.000																	
14. WI133 (Patient 3)	0.073	0.060	0.067	0.069	0.058	0.043	0.044	0.066	0.061	0.080	0.062	0.000	0.000																
15. MN14-1539 (Patient 5)	0.072	0.058	0.062	0.065	0.055	0.051	0.049	0.062	0.058	0.078	0.060	0.009	0.009	0.009															
16. MN14-1420 (Patient 6)	0.072	0.058	0.062	0.065	0.055	0.052	0.050	0.063	0.059	0.078	0.060	0.009	0.009	0.009	0.000														
17. *B. valaisiana* VS116	0.078	0.085	0.089	0.073	0.082	0.076	0.075	0.075	0.085	0.086	0.084	0.077	0.077	0.077	0.077	0.077													
18. *B. californiensis* CA446	0.076	0.055	0.059	0.077	0.046	0.053	0.053	0.076	0.049	0.083	0.053	0.058	0.058	0.058	0.055	0.054	0.080												
19. *B. chilensis* VA1	0.095	0.109	0.112	0.100	0.106	0.105	0.105	0.100	0.109	0.101	0.111	0.099	0.099	0.099	0.097	0.097	0.105	0.107											
20. *B. hermsii* DAH	0.286	0.298	0.298	0.300	0.294	0.294	0.292	0.302	0.289	0.290	0.300	0.290	0.290	0.290	0.288	0.287	0.291	0.282	0.285										
21. *B. japonica* HO14	0.067	0.086	0.086	0.068	0.079	0.077	0.075	0.068	0.080	0.078	0.085	0.074	0.074	0.074	0.073	0.072	0.080	0.077	0.099	0.288									
22. *B. miyamotoi* HT31	0.294	0.303	0.307	0.302	0.297	0.303	0.302	0.306	0.295	0.296	0.301	0.296	0.296	0.296	0.295	0.294	0.302	0.290	0.297	0.125	0.295								
23. *B. miyamotoi* M1029	0.293	0.303	0.305	0.303	0.298	0.305	0.303	0.306	0.295	0.297	0.299	0.297	0.297	0.297	0.296	0.296	0.299	0.292	0.298	0.129	0.295	0.019							
24. *B. sinica* CMN3	0.084	0.102	0.103	0.080	0.097	0.094	0.094	0.086	0.099	0.093	0.102	0.087	0.087	0.087	0.086	0.086	0.096	0.095	0.110	0.291	0.078	0.294	0.293						
25. *B. persica* No12	0.295	0.304	0.299	0.300	0.302	0.301	0.299	0.301	0.301	0.293	0.304	0.292	0.292	0.292	0.291	0.291	0.287	0.298	0.297	0.155	0.294	0.170	0.177	0.296					
26. *B. tanukii* Hk501	0.074	0.084	0.088	0.067	0.079	0.076	0.074	0.067	0.079	0.082	0.083	0.070	0.070	0.070	0.071	0.071	0.051	0.080	0.102	0.294	0.071	0.305	0.303	0.087	0.288				
27. *B. carolinensis* SCW-22	0.086	0.061	0.063	0.083	0.027	0.058	0.058	0.083	0.032	0.089	0.061	0.061	0.061	0.061	0.057	0.058	0.087	0.047	0.109	0.294	0.078	0.305	0.304	0.097	0.301	0.082			
28. *B. lusitaniae* PoTiB2	0.070	0.080	0.082	0.066	0.078	0.075	0.076	0.067	0.082	0.077	0.079	0.071	0.071	0.071	0.070	0.070	0.073	0.075	0.096	0.290	0.071	0.300	0.301	0.086	0.298	0.074	0.081		
29. *B. turdi* Ya501	0.076	0.089	0.094	0.072	0.084	0.083	0.083	0.075	0.085	0.085	0.090	0.080	0.080	0.080	0.078	0.078	0.082	0.087	0.102	0.288	0.080	0.292	0.293	0.089	0.293	0.078	0.086	0.078	

Supplemental Table 3. Pairwise genetic distances of 8 concatenated housekeeping genes from patient isolates (MN14-1420, MN14-1539) (highlighted in blue) as compared to 18 Bbsl genospecies (no highlight) and 3 RF species (no highlight). Genetic distances were calculated in MEGA 5.0 using the Kimura-2 model. Percent genetic similarity is calculated using the formula: 100 − (genetic distance × 100). The lowest pairwise genetic distance, highest genetic similarity for the patient isolates (MN14-1420, MN14-1539) is to *B. burgdorferi* (0.051 to 0.048; 94.9% and 95.2% similarity) (highlighted in green). The highest pairwise genetic distance, lowest similarity for the patient isolates (MN14-1420, MN14-1539) is to RF borreliae (0.300 to 0.292; 70% to 70.8) (highlighted in red).

Strains	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
1. *B. afzelii* VS461																																									
2. *B. afzelii* PKo	0.002																																								
3. *B. americana* SCW-30h	0.083	0.083																																							
4. *B. americana* SCW-41	0.085	0.086	0.015																																						
5. *B. andersonii* 21123	0.080	0.080	0.054	0.053																																					
6. *B. bavariensis* PBi	0.064	0.065	0.081	0.082	0.081																																				
7. *B. bavariensis* PZwi	0.064	0.065	0.081	0.083	0.081	0.000																																			
8. *B. bissetti* DN127	0.079	0.080	0.060	0.059	0.062	0.079	0.079																																		
9. *B. burgdorferi* 94a	0.076	0.077	0.048	0.051	0.055	0.077	0.077	0.057																																	
10. *B. burgdorferi* 297	0.074	0.075	0.048	0.050	0.053	0.076	0.076	0.056	0.004																																
11. *B. burgdorferi* B31	0.076	0.077	0.049	0.051	0.054	0.076	0.077	0.056	0.004	0.002																															
12. *B. burgdorferi* B348	0.075	0.076	0.047	0.048	0.056	0.078	0.078	0.057	0.009	0.006	0.008																														
13. *B. burgdorferi* B485	0.076	0.077	0.048	0.051	0.055	0.077	0.077	0.057	0.000	0.004	0.004	0.009																													
14. *B. burgdorferi* BL522	0.074	0.075	0.047	0.048	0.055	0.078	0.078	0.057	0.007	0.006	0.008	0.005	0.007																												
15. *B. burgdorferi* ca92-1096	0.076	0.077	0.048	0.050	0.055	0.078	0.079	0.057	0.008	0.004	0.006	0.007	0.008	0.008																											
16. *B. burgdorferi* ca92-1337	0.075	0.076	0.047	0.048	0.055	0.078	0.078	0.057	0.007	0.006	0.008	0.005	0.007	0.000	0.008																										
17. *B. burgdorferi* MC104	0.075	0.076	0.048	0.050	0.053	0.076	0.077	0.056	0.004	0.001	0.003	0.007	0.004	0.006	0.005	0.006																									
18. *B. burgdorferi* MC115	0.076	0.078	0.049	0.051	0.054	0.077	0.078	0.057	0.004	0.003	0.004	0.007	0.004	0.007	0.006	0.007	0.003																								
19. *B. burgdorferi* MC138	0.075	0.076	0.048	0.050	0.055	0.076	0.076	0.057	0.006	0.005	0.005	0.004	0.006	0.005	0.005	0.006	0.005	0.005																							
20. *B. burgdorferi* NE49	0.075	0.076	0.049	0.052	0.052	0.075	0.075	0.057	0.017	0.015	0.017	0.017	0.017	0.017	0.017	0.016	0.017	0.016	0.015																						
21. *B. burgdorferi* Z41293	0.076	0.076	0.048	0.050	0.052	0.076	0.077	0.057	0.017	0.015	0.016	0.017	0.017	0.017	0.016	0.017	0.015	0.015	0.015	0.007																					
22. *B. carolinensis* SCW-22	0.084	0.085	0.060	0.061	0.063	0.084	0.084	0.024	0.058	0.057	0.058	0.058	0.058	0.059	0.058	0.059	0.058	0.058	0.058	0.056	0.057																				
23. *B. garinii* 20047	0.065	0.066	0.081	0.083	0.079	0.020	0.020	0.078	0.075	0.074	0.075	0.076	0.075	0.076	0.077	0.076	0.075	0.076	0.074	0.074	0.075	0.084																			
24. *B. japonica* HO14	0.067	0.068	0.087	0.087	0.085	0.067	0.067	0.080	0.078	0.076	0.077	0.079	0.078	0.078	0.079	0.079	0.076	0.077	0.078	0.078	0.076	0.080	0.068																		
25. *B. kurtenbachii* 25015	0.082	0.083	0.063	0.064	0.065	0.082	0.082	0.035	0.060	0.058	0.059	0.060	0.060	0.060	0.060	0.060	0.059	0.060	0.059	0.058	0.060	0.030	0.081	0.081																	
26. *B. kurtenbachii* IL96-255	0.081	0.082	0.062	0.063	0.064	0.080	0.080	0.035	0.060	0.058	0.058	0.059	0.060	0.060	0.059	0.060	0.059	0.059	0.058	0.058	0.060	0.031	0.080	0.081	0.005																
27. *B. lusitaniae* PoTiB2	0.067	0.068	0.080	0.082	0.080	0.066	0.065	0.078	0.076	0.074	0.076	0.075	0.076	0.075	0.075	0.075	0.075	0.076	0.075	0.075	0.076	0.082	0.067	0.070	0.082	0.081															
28. *B. sinica* CMN3	0.081	0.082	0.103	0.106	0.103	0.080	0.080	0.098	0.095	0.095	0.095	0.096	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.096	0.095	0.099	0.086	0.080	0.100	0.100	0.085														
29. *B. spielmanii* PsigII27/6	0.066	0.065	0.088	0.089	0.086	0.075	0.075	0.088	0.082	0.081	0.082	0.082	0.082	0.081	0.082	0.081	0.082	0.082	0.081	0.083	0.082	0.090	0.076	0.078	0.090	0.089	0.076	0.093													
30. *B. tanukii* Hk501	0.072	0.073	0.085	0.084	0.086	0.066	0.066	0.080	0.077	0.075	0.077	0.076	0.077	0.076	0.077	0.076	0.076	0.076	0.075	0.075	0.074	0.083	0.066	0.071	0.082	0.080	0.071	0.087	0.080												
31. *B. turdi* Ya501	0.075	0.076	0.090	0.089	0.092	0.071	0.072	0.083	0.084	0.082	0.083	0.083	0.084	0.082	0.084	0.082	0.083	0.084	0.083	0.083	0.083	0.085	0.073	0.080	0.087	0.085	0.078	0.089	0.084	0.076											
32. *Borrelia* sp. SCW-30a	0.083	0.083	0.018	0.008	0.054	0.083	0.083	0.060	0.051	0.050	0.051	0.048	0.051	0.048	0.050	0.048	0.050	0.051	0.050	0.050	0.048	0.061	0.084	0.087	0.064	0.063	0.080	0.104	0.089	0.084	0.090										
33. Patient 5 (MN14-1539)	0.070	0.071	0.057	0.058	0.059	0.066	0.067	0.053	0.051	0.049	0.050	0.050	0.051	0.050	0.050	0.050	0.050	0.051	0.050	0.049	0.048	0.056	0.065	0.073	0.057	0.056	0.069	0.088	0.078	0.071	0.077	0.058									
34. Patient 6 (MN14-1420)	0.070	0.072	0.057	0.058	0.059	0.067	0.067	0.053	0.051	0.049	0.051	0.051	0.051	0.050	0.051	0.051	0.050	0.051	0.050	0.049	0.049	0.056	0.065	0.073	0.058	0.056	0.069	0.088	0.078	0.071	0.077	0.058	0.000								
35. *B. valaisiana* VS116	0.075	0.076	0.086	0.087	0.088	0.070	0.070	0.083	0.078	0.077	0.078	0.078	0.078	0.077	0.078	0.078	0.077	0.077	0.076	0.078	0.077	0.087	0.072	0.079	0.087	0.086	0.072	0.095	0.083	0.050	0.081	0.086	0.078	0.077							
36. *B. californiensis* CA446	0.075	0.075	0.054	0.054	0.057	0.075	0.075	0.044	0.052	0.050	0.051	0.053	0.052	0.053	0.052	0.053	0.051	0.053	0.052	0.051	0.050	0.045	0.075	0.078	0.047	0.046	0.074	0.095	0.083	0.080	0.084	0.052	0.052	0.052	0.081						
37. *B. chilensis* VA1	0.093	0.094	0.110	0.113	0.112	0.098	0.098	0.106	0.107	0.106	0.107	0.107	0.107	0.107	0.108	0.107	0.107	0.107	0.106	0.104	0.106	0.108	0.098	0.098	0.109	0.108	0.093	0.109	0.101	0.101	0.101	0.113	0.097	0.097	0.101	0.106					
38. *B. hermsii* DAH	0.290	0.290	0.300	0.304	0.302	0.303	0.303	0.296	0.297	0.295	0.296	0.298	0.297	0.299	0.295	0.298	0.295	0.296	0.297	0.293	0.295	0.296	0.306	0.291	0.292	0.292	0.292	0.293	0.294	0.298	0.292	0.302	0.292	0.292	0.294	0.286	0.287				
39. *B. miyamotoi* HT31	0.297	0.299	0.308	0.307	0.311	0.306	0.306	0.303	0.309	0.305	0.307	0.310	0.309	0.310	0.307	0.310	0.306	0.308	0.309	0.304	0.305	0.310	0.312	0.299	0.300	0.300	0.303	0.297	0.301	0.310	0.299	0.305	0.300	0.300	0.306	0.295	0.300	0.125			
40. *B. miyamotoi* M1029	0.295	0.297	0.307	0.305	0.309	0.306	0.306	0.303	0.309	0.306	0.307	0.310	0.309	0.311	0.306	0.310	0.306	0.309	0.309	0.305	0.305	0.308	0.311	0.299	0.300	0.300	0.304	0.296	0.301	0.307	0.298	0.303	0.300	0.300	0.303	0.296	0.299	0.130	0.019		
41. *B. persica* No12	0.298	0.299	0.306	0.304	0.301	0.302	0.302	0.305	0.303	0.300	0.302	0.303	0.303	0.303	0.301	0.302	0.299	0.301	0.303	0.299	0.300	0.304	0.304	0.297	0.305	0.304	0.301	0.299	0.295	0.291	0.298	0.305	0.296	0.295	0.290	0.301	0.300	0.156	0.173	0.181	

Supplemental Table 4. Melting temperatures (Tm) for Bbsl *oppA1* PCR products accurately differentiates Candidatus *Borrelia mayonii* from 15 other Bbsl genospecies and does not detect members of the RF group.*

Organism	Source of Material (Accession number)	Tm (°Celsius)
***Borrelia burgdorferi* sensu lato genospecies**		
Borrelia afzelii	DSMZ (16073)	53.83
Borrelia afzelii	ATCC (51992)	54.58
Borrelia americana	ATCC (BAA-1877)	54.36
Borrelia andersonii	ATCC (700555)	56.95
Borrelia bavariensis	DSMZ (DSM 23469)	54.97
Borrelia bissettii	DSMZ (17990)	55.94
Borrelia burgdorferi	ATCC (51990)	63.60
Borrelia burgdorferi	ATCC (53899)	64.91
Borrelia burgdorferi	ATCC (35210)	64.90
Borrelia burgdorferi	ATCC (55131)	64.13
Borrelia californiensis	DSMZ (17989)	59.20
Borrelia carolinensis	ATCC (BAA-1773)	57.62
Borrelia garinii	ATCC (51991)	54.82
Borrelia garinii	ATCC (51383)	54.99
Borrelia japonica	ATCC (51557)	NEGATIVE
Borrelia kurtenbachii	ATCC (BAA 2495)	58.40
Candidatus *Borrelia mayonii*	Patient specimens	60.38 – 61.24**
Borrelia sinica	DSMZ (DSM 23262)	NEGATIVE
Borrelia spielmanii	DSMZ (DSM 16813)	NEGATIVE
Borrelia valaisiana	DSMZ (DSM 21467)	52.30
Borrelia yangtze	DSMZ (DSM 24625)	58.03
Relapsing Fever borreliae		
Borrelia coriaceae	ATCC 43381	NEGATIVE
Borrelia duttoni	CP000976***	NEGATIVE
Borrelia hermsii	DSMZ 5251	NEGATIVE
Borrelia miyamotoi	CDC FR64b	NEGATIVE
Borrelia miyamotoi	CDC HT31	NEGATIVE
Borrelia miyamotoi	CDC HT24	NEGATIVE
Borrelia miyamotoi	MDH A4 Tick	NEGATIVE
Borrelia parkeri	CDC MT90-900	NEGATIVE
Borrelia recurrentis	CP000993***	NEGATIVE
Borrelia recurrentis	CDC 99-0708A	NEGATIVE
Borrelia turcica	DSMZ 16138	NEGATIVE
Borrelia turicatae	CDC MT90-901	NEGATIVE

* This assay does not detect relapsing fever borreliae, including *Borrelia miyamotoi*

** Range of melting temperatures observed for the 6 patient specimens

Abbreviations: ATCC – American Type Culture Collection, CDC – Centers for Disease Prevention and Control, DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen Gmbh (German Collection of Microorganisms and Cell Cultures), MDH – Minnesota Department of Health, Tm – melting temperature

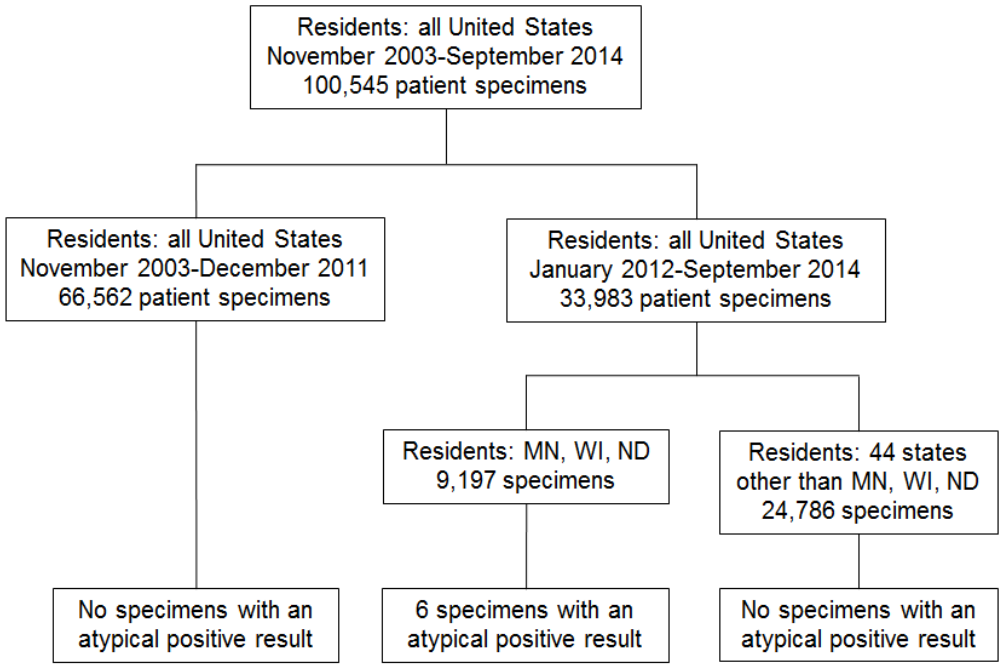
*** Specimens obtained from the Collection de Souches de l'Unite des Rickettsies, Marseilles, France

Supplemental Table 5. Results of polymerase chain reaction testing of *Ixodes scapularis* ticks collected in two Wisconsin counties, 2009-2014*

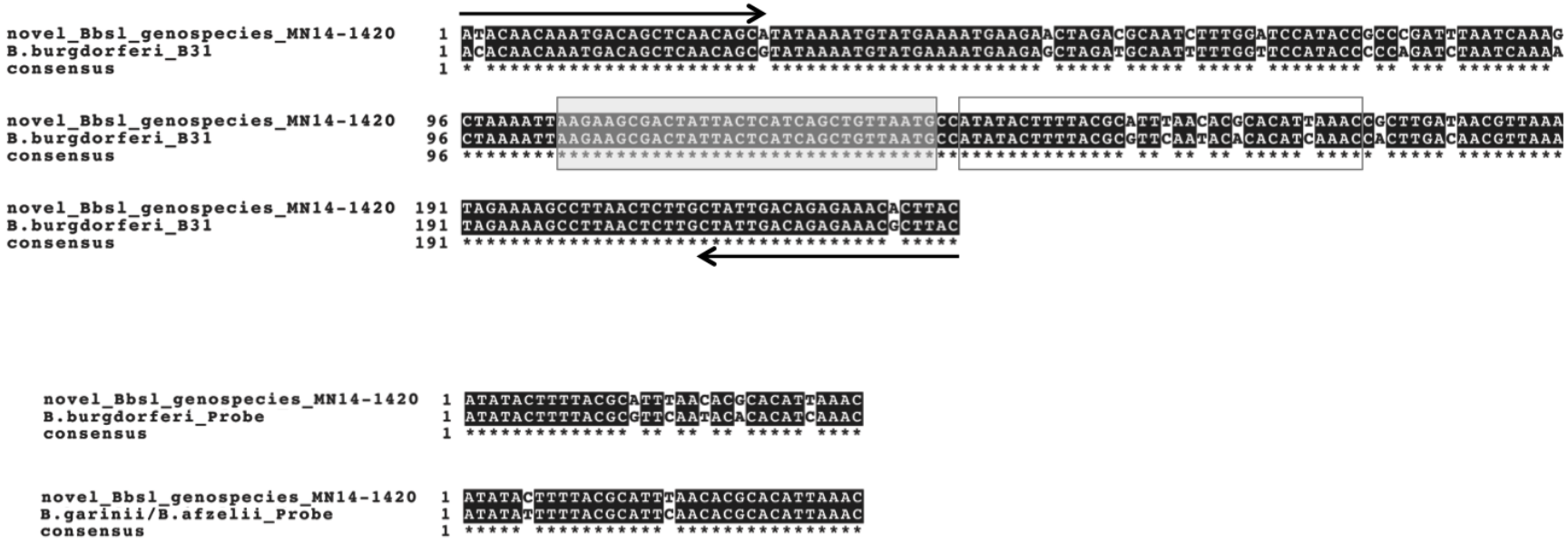
Collection Site County	Collection Date	Adult *I. scapularis* PCR results		Nymphal *I. scapularis* PCR results		Total PCR results	
		Novel *Borrelia* sp. No. Pos/Total (%)**	*Borrelia burgdorferi* No. Pos/Total (%)**	Novel *Borrelia* sp. No. Pos/Total (%)***	*Borrelia burgdorferi* No. Pos/Total (%)**	Novel *Borrelia* sp. No. Pos/Total (%)**	*Borrelia burgdorferi* No. Pos/Total (%)**
Barron	October 2013	1/170 (0.6) (1 male)	68/170 (40.0)	NC	NC	1/170 (0.6)	68/170 (40.0)
Barron	June-July 2014	14/267 (5.2)*** (11 females, 3 males)	89/267 (33.3) [b]	3/81 (3.7)	22/81 (27.1)	17/348 (4.9)	111/348 (31.9)
Eau Claire	2009-2010	0/28 (0)	5/28 (17.9)	1/112 (0.9)	11/112 (9.8)	1/140 (0.7)	16/140 (11.4)
	Total	15/465 (3.2)	162/465 (34.8)	4/193 (2.1)	33/193 (17.1)	19/658 (2.9)	195/658 (29.6)

*Abbreviations: PCR - polymerase chain reaction, Pos – positive, NC - not collected (nymphs were not found during this collection), No. - number
**Number positive divided by the total number tested and percentage
***Two adult ticks were co-infected with both agents

Supplemental Figures

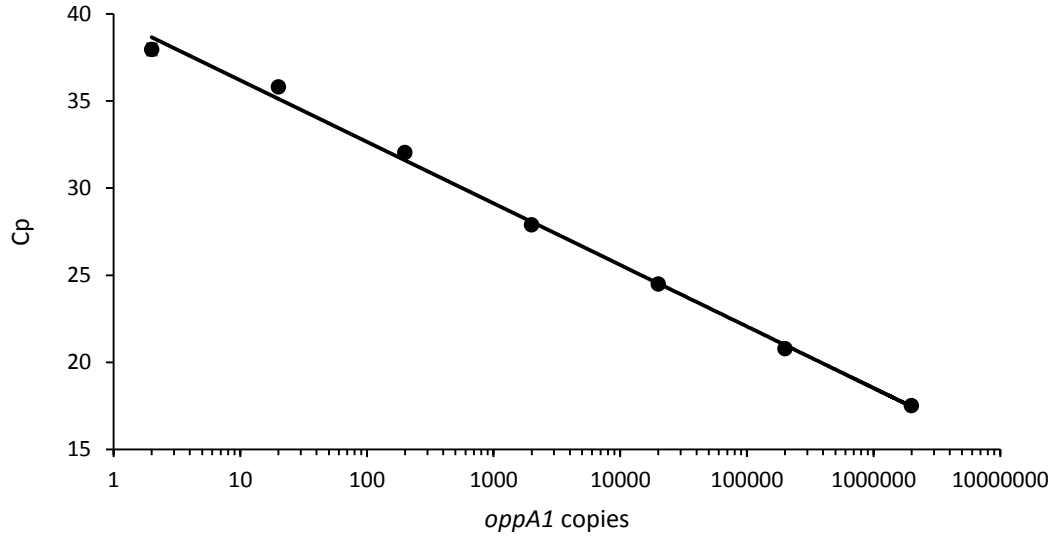


Supplemental Figure 1. Number of diagnostic patient specimens tested by Mayo Medical Laboratories during November 2003-September 2014 using *oppA1* PCR and the number of atypical (novel Bbsl genospecies) results. Abbreviations: Bbsl – *Borrelia burgdorferi sensu lato*, MN – Minnesota, ND – North Dakota, PCR – polymerase chain reaction, WI – Wisconsin

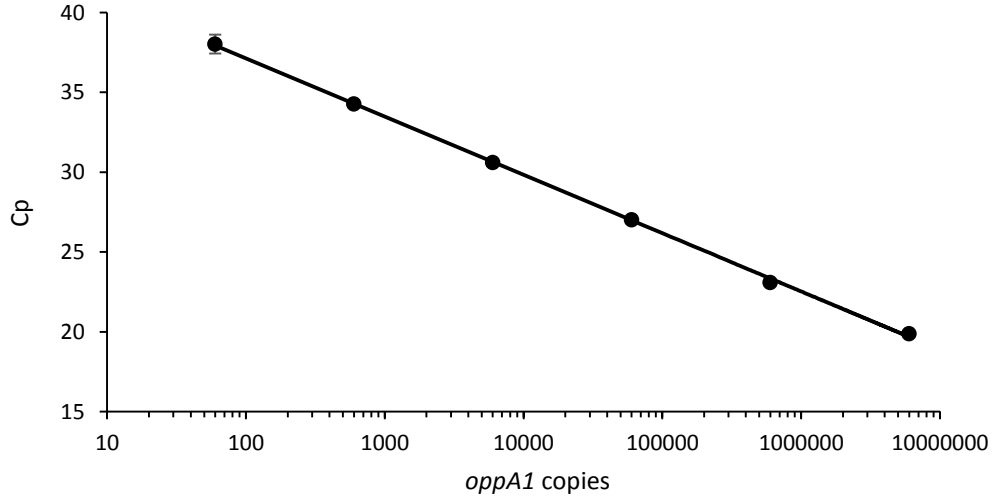


Supplemental Figure 2. Alignment of the *oppA1* PCR amplicon for the novel Bbsl genospecies MN14-1420 as compared to *B. burgdorferi* B31. Highlighted sequence and astericks indicate nucleotide identity. The *oppA1* forward and reverse primers are indicated by black arrows, the FL-probe sequence binding site is indicated by the shaded grey box, and the species specific 640-probe binding site is indicated by the unshaded grey box. Lower panels show the alignment of the *oppA1* genospecies-specific 640-probe (*B. burgdorferi* and *B. garinii/B afzelii*) as compared to the novel Bbsl genospecies (MN14-1420) sequence.

A.

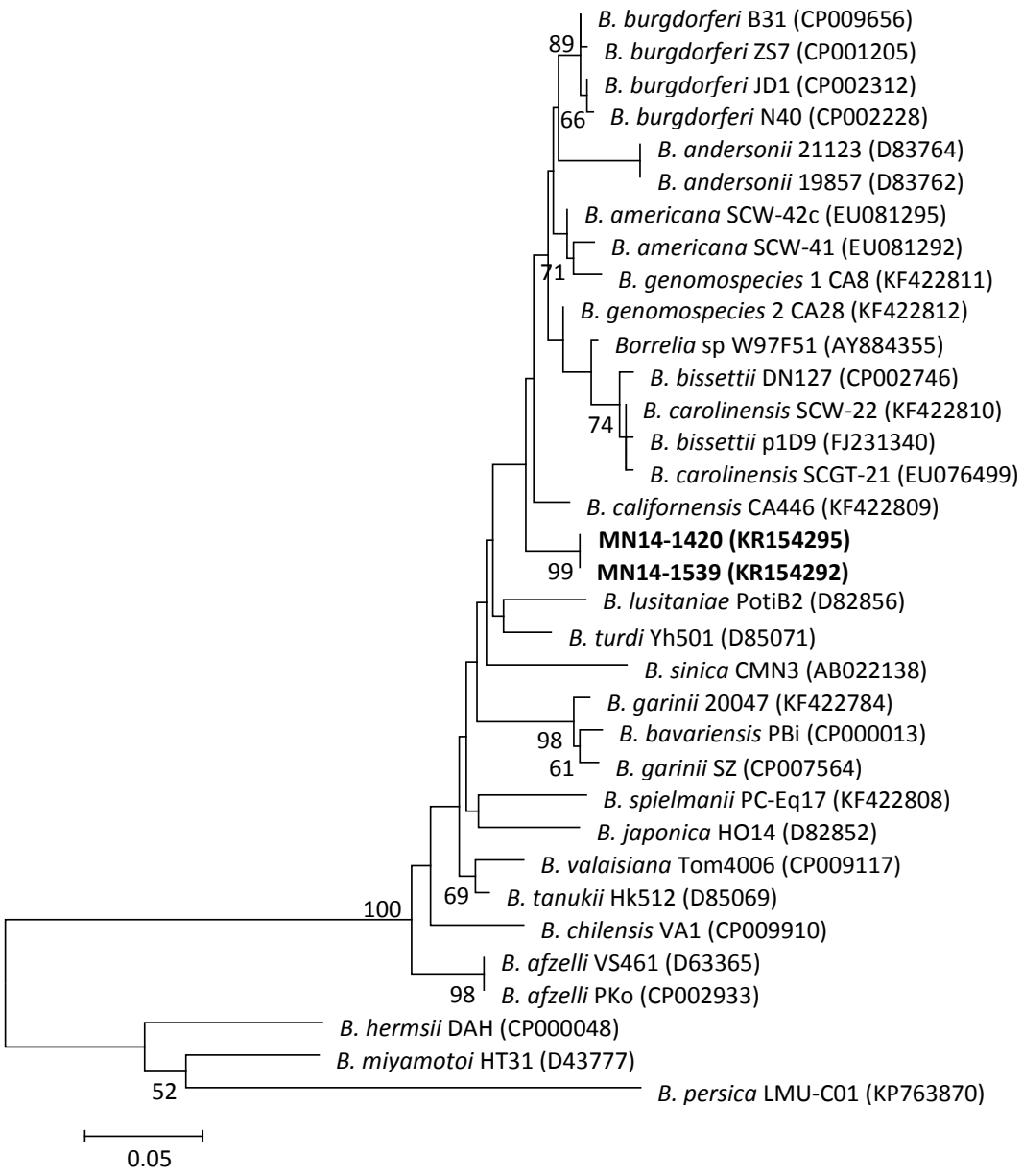


B.

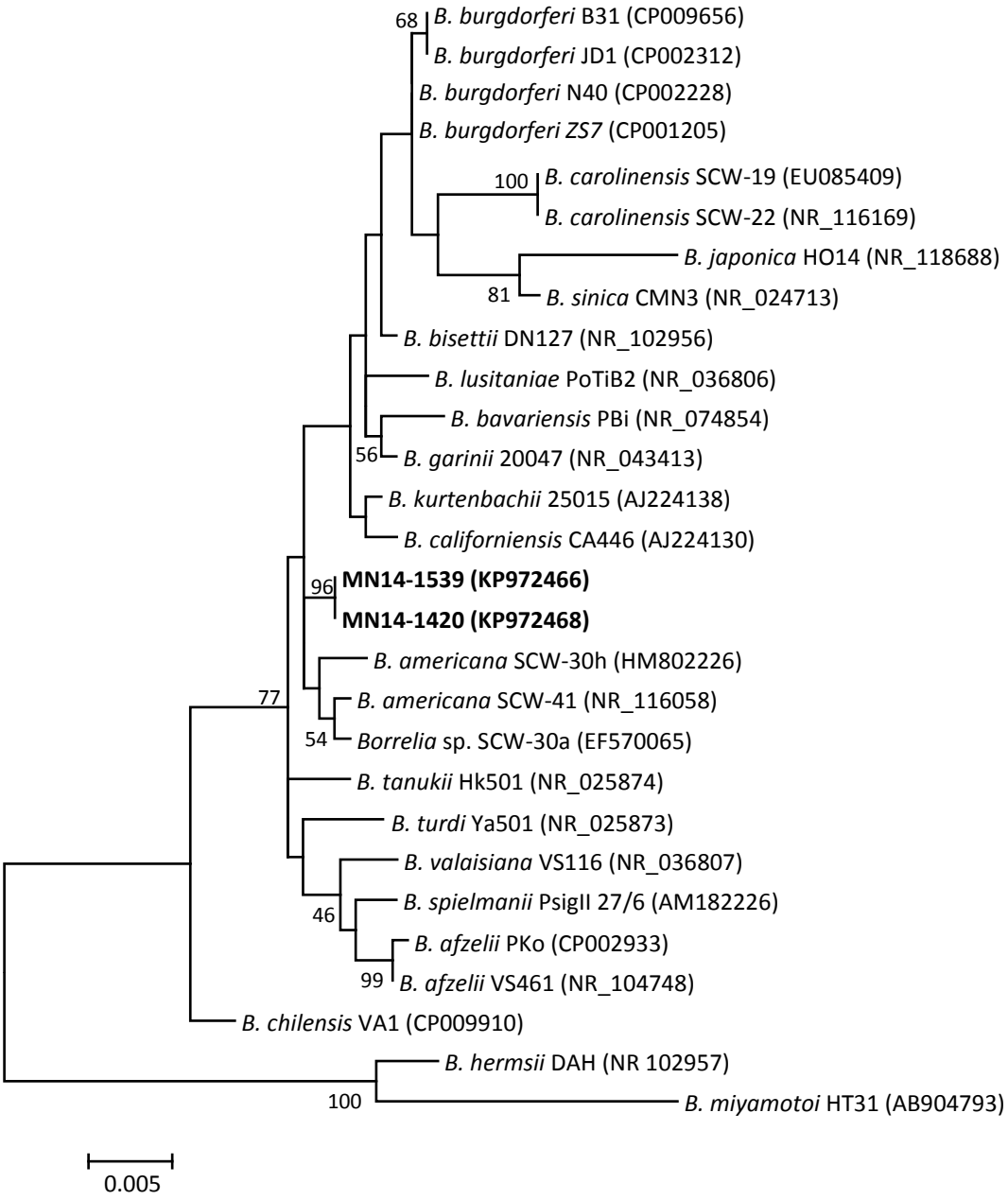


Supplemental Figure 3. Standard curves relating the number of *oppA1* copies in A) *B. burgdorferi* and the B) novel *Bbsl* genospecies to the crossing point (Cp), determined by *oppA1* real-time PCR. The *oppA1* gene is present in single copy on the chromosome of both species.

A.


B. burgdorferi B31 (CP009656)
89
B. burgdorferi ZS7 (CP001205)
B. burgdorferi JD1 (CP002312)
66
B. burgdorferi N40 (CP002228)
B. andersonii 21123 (D83764)
B. andersonii 19857 (D83762)
B. americana SCW-42c (EU081295)
B. americana SCW-41 (EU081292)
71
B. genomospecies 1 CA8 (KF422811)
B. genomospecies 2 CA28 (KF422812)
Borrelia sp W97F51 (AY884355)
B. bissettii DN127 (CP002746)
B. carolinensis SCW-22 (KF422810)
74
B. bissettii p1D9 (FJ231340)
B. carolinensis SCGT-21 (EU076499)
B. californensis CA446 (KF422809)
MN14-1420 (KR154295)
99
MN14-1539 (KR154292)
B. lusitaniae PotiB2 (D82856)
B. turdi Yh501 (D85071)
B. sinica CMN3 (AB022138)
B. garinii 20047 (KF422784)
98
B. bavariensis PBi (CP000013)
61
B. garinii SZ (CP007564)
B. spielmanii PC-Eq17 (KF422808)
B. japonica HO14 (D82852)
B. valaisiana Tom4006 (CP009117)
69
B. tanukii Hk512 (D85069)
100
B. chilensis VA1 (CP009910)
B. afzelli VS461 (D63365)
98
B. afzelli PKo (CP002933)
B. hermsii DAH (CP000048)
B. miyamotoi HT31 (D43777)
52
B. persica LMU-C01 (KP763870)
0.05

B.



Supplemental Figure 4. Phylogenetic analysis of a (A) 392 bp fragment of the *flaB* gene and (B) 1280 bp fragment of the 16s rRNA gene from patient isolates (MN14-1539, MN14-1420) as compared with Bbsl and RF species. Accession numbers are indicated for sequences retrieved from GenBank. Bootstrap support values >50% are shown. The scale bar corresponds to (A) 0.05 or (B) 0.005 substitutions per nucleotide position.

Supplemental Figure 5. Phylogenetic analysis of 7 concatenated housekeeping genes: *uvrA*, *rplB*, *recG*, *pyrG*, *pepX*, *clpX*, and *clpA* amplified from patient (MN14-1539, MN14-1420, ND132, WI133) and tick (EC10N1) specimens as compared to 18 different Bbsl genospecies and 3 RF species. Bootstrap support values >50% are shown. The scale bar corresponds to 0.05 substitutions per nucleotide position. The source of other *Borrelia* gene sequences is indicated in the supplemental methods.

Supplemental Methods

***oppA1* Standard Curves** - Genomic DNA was purified from cultures of *B. burgdorferi* B31 and the novel Bbsl genospecies MN-1420 using the QIAamp DNA Mini Kit (Qiagen), including RNAse treatment. Ten-fold dilutions were prepared based on genome equivalents for each genospecies as determined by measurement of the DNA concentrations (optical density at 260 nm), using known genome sizes of 1.5 Mb for *B. burgdorferi* and 1.4 Mb for the novel Bbsl genospecies and the fact that the *oppA1* gene is single copy in both organisms (based on whole genome sequencing data; data not shown). Therefore one *oppA1* copy equates to 1 genome equivalent. One genome equivalent is ~1.6 fg for *B. burgdorferi* B31 and ~1.5 fg for MN-1420. Each dilution was tested in triplicate using the *oppA1* real-time PCR assay and the standard error determined. Estimates of *oppA1* copy numbers for patient blood specimens were calculated using the *oppA1* Cp values for the blood specimens and the standard curves shown in Fig. S3. The estimated copy numbers do not account for run-to-run variation, as standard curve comparisons were performed subsequent to specimen testing.

Multilocus Sequence Typing – Housekeeping gene sequences used are as follows: for *B. afzelii* PKo, *B. burgdorferi* B31, and *B. bissettii* DN127 GenBank accession numbers were NC_017238, NC_001318, and CP002746, respectively; for *B. americana* SCW-30h GenBank sequences were JN805988, JN805980, JN805972, JN805964, JN704339, JN704323, JN704315; for *Borrelia* sp. SCW-30a GenBank sequences were JN805981, JN805973, JN805965, JN805957, JN704332, JN704316, JN704308; for *B. bavariensis* PZwi GenBank sequences were KC833677, KC833658, KC833639, KC833620, KC833601, KC833563, KC833544; for *B. spielmanii* PsigII 27/6 GenBank sequences were AB526162, AB526161, AB526160, AB526159, AB526158, AB526156, AB526155; for *B. kurtenbachii* IL96-255 GenBank sequences were KF052030, KF052026, KF052022, KF052017, KF052014, KF052005, KF052002; for *B. valaisiana* Am501 GenBank sequences were AB526130, AB526129, AB526128, AB526127, AB526126, AB526124, AB526123 and for *B. andersonii* 21123 GenBank sequences were AB526122, AB526121, AB526120, AB526119, AB526118, AB526116, AB526115. Housekeeping sequences from all other *Borrelia* included in the 7 and 8 gene MLSA were retrieved from http://pubmlst.org/borrelia/.

Microscopic Quantification – Wet mounts (4) were prepared by pipetting 5 µl of diluted *B. burgdorferi* B31 grown in Barbour-Stoenner-Kelly media onto a glass slide and covering with a 22 mm x 22 mm coverslip. The number of spirochetes per field (20 per slide) were counted using a Zeiss AkioScope (40X objective and 10X ocular) and averaged for the 4 wet mounts. The number of spirochetes was also determined using the same *B. burgdorferi* B31 suspension and a Petroff-Hausser counting chamber. A conversion factor was determined between the number of organisms per field determined by wet mount and the spirochete number determined using the Petroff-Hausser counting chamber and this conversion factor used to determine the number of spirochetes in wet mounts prepared from diluted patient blood.

Serologic testing: First tier serology assays utilized included the C6 *B. burgdorferi* (Lyme) ELISA™ assay (Immunetics) and whole-cell sonicate EIA (bioMérieux). Second tier assays included the *Borrelia* B31 IgM and IgG ViraStripe® assays (Viramed Biotech AG) and IgM and IgG immunoblots (MarDx Diagnostics). Tests were performed according to the manufacturer's instructions.

Culture: An aliquot of patient's blood (50 ul) was placed into 4·5 ml of modified Barbour-Stoenner-Kelly medium and incubated under microaerophilic conditions at 34°C. Inoculated cultures were blind passaged after six to seven days by transferring 50 ul of the original culture to 4.5 ml of fresh medium.

Tick Collection and Processing: *Ixodes scapularis* ticks were collected at approximate sites of possible patient exposure in Barron County, Wisconsin, during September and October 2013 and June-July 2014. Ticks were collected by dragging a 1 m^2 fabric cloth across vegetation in mixed hardwood forest and along trails and adjacent grassy fields. Ticks were identified to life stage and species according to published keys (Keirans and Litwak 1989). Ticks collected during 2013 were minced and digested in proteinase K, followed by DNA extraction using the MagNA Pure 2.0 (Roche) and *oppA1* PCR. Ticks collected during 2014 were first submerged in hydrogen peroxide followed by 70% ethanol.